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DIFFERENCE IN AGE-DEPENDENT GENE EXPRESSIONS IN THE EARLY STAGE OF PARTIAL-THICKNESS ARTICULAR CARTILAGE DEFECT IN RAT

N. Ikegawa, R. Akagi, M. Saito, H. Tsuruoka, S. Yamaguchi, K. Matsuki, K. Nakagawa, T. Sasho, K. Takahashi
Chiba Univ. Hosp., Chiba, Japan

Purpose: The partial-thickness (PT) articular cartilage defects do not to heal in mature animals whereas those of immature animals potentially heal. The purpose of this study is to elucidate differences in repair process in PT cartilage defects between mature and immature animals.
Methods: The partial-thickness (PT) articular cartilage defects were created on the medial femoral condyles of 3-week-old (immature) and 16-week-old (mature) SD rats (male). Specially designed chisel were used to create 100-micrometer-depth defects which was about 50% depth of mature cartilage. Contra-lateral knees were sham-operated.
For histologic analysis, animals were euthanized 6 weeks after surgery. The condyles were stained with hematoxylin-eosin, safranine O/fast green and toluidine blue. Repair sites were evaluated using the Mankin score.
To examine different response to PT cartilage injury between immature and mature cartilage, micro-array technique was employed. Genes induced by PT cartilage injury (GIPT) in immature animals were identified by comparing gene expressions between 3-week PT cartilage and 3-week control cartilage. GIPT of mature animals were identified as well. Then the GIPT of immature and mature was compared. For this study animals were euthanized 24 hours after creating PT defects and cartilage RNA was extracted for further process.
Data were analyzed using a two- tailed Mann-Whitney U test to compare difference in Mankin score. $P<0.05$ was considered significant.
Results: *Macroscopical features.* Five of six knees in immature defect group had obscure border between defects and surrounding cartilage, which was indicative of cartilage repair. Two of six knees in mature defect group had fissures and other 4 knees had clear defined border between defects and surrounding cartilage.
Microscopical features. Histologic analysis revealed that defects were repaired well at 6 weeks in the immature defect group, though diminished stainability of safranine-O was observed in the repaired site comparing to normal cartilage. In the mature defect group, the defect was filled by fibrous cells, lowly-cellular and decreased safranine-O stainability.
Average of the Mankin score was 0.5 in the immature sham group, 2.8 in the immature defect group, 1.5 in the mature sham group, and 9.7 in the mature defect group. We found higher scores in both of immature (p=0.005) and mature defect groups (p=0.003) compared with sham groups. The immature defect group scored obviously higher than the mature defect group (p=0.003). The scores of the immature defect group were lower than that of the mature defect group in each item of the Mankin parameters.
Micro-array. No interleukins were induced by PT cartilage defects in immature cartilage whereas IL1-beta and IL-6 were induced in mature cartilage. Among immediate early genes, *Fos* and *ATF-3* were induced only in mature cartilage. No differences in expression levels of family of transforming growth factor beta (TGF-beta) were found between the two group.
Conclusions: PT cartilage injury of 3-week old rat knee was spontaneously repaired but that of 16-week old was not. Lack of immediate response to cartilage damage might account for this difference.

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LONG-TERM STUDY OF STRATIFIED ALGINATE CONSTRUCT FOR CARTILAGE TISSUE ENGINEERING

J. Schiavi-Tritz[1], N. Charif[1], N. de Isla[1], R. Rahouadj[2], A. Pinzano[1], D. Bensoussan[1,3], N. Benkirane-Jessel[4,5], J. Magdalou[1], J.-F. Stoltz[1,3], C. Huselstein[1]
[1]CNRS - UMR 7561 -Nancy Université, Faculté de Médecine, Vandoeuvre les Nancy, France; [2]CNRS - UMR 7563 - LEMTA INPL, Vandoeuvre les Nancy, France; [3]CHU NANCY – Unité de Thérapie Cellulaire et Tissulaire, Vandoeuvre les Nancy, France; [4]INSERM - UMR 977, Faculté de Médecine, Strasbourg, France; [5]CHU NANCY – COT, Vandoeuvre les Nancy, France

Objective: Cartilage defect self-regeneration gives a fibro-tissue with weak mechanical properties. Until now, most of research ways product homogenous scaffolds without mimicking cartilage morphology, yet, stratified Cartilage Tissue Engineering (CTE) is developing to obtain strata in scaffolds. Our work aimed at contributing to stratified CTE with providing an original build-up method to obtain scaffolds composed with sodium Alginate and Hyaluronic Acid (Alg/HA) seeded with human Mesenchymal Stem Cells (hMSCs).
Methods: Therefore, a stratified hydrogel was build up by a simple and progressive spray of cellular hydrogel suspension alternate with polyelectrolyte multilayer to weld strata. hMSCs were embedded in Alg/HA gel, then sprayed and the hydrogel was gelled in a bath of CaCl2. We evaluated cells comportment in layered structure in long-term culture (56 days) with a differentiated medium, without growth factor, usually used in CTE. In fact, we followed matrix synthesis at 28 days of culture up to 56 days to evaluate qualitative proteic expression of hMSCs thanks to histological and confocal microscopy observations. Finally, we compared quantitative gene expression of hMSCs cultured in vitro in the scaffold during 28 and 56 days.
Results: We explored the ability to construct bilayered scaffold with hydrogel strata welded by polyelectrolyte multilayer with a high of 2mm. One notice that we observed good cohesion during in vitro culture. Moreover, multilayers avoid a mix of strata during the build-up. Furthermore, matrix synthesis was followed up to 56 days of in vitro culture with histological slices staining and type I, II and X collagen labeling for confocal observation. At 3 days of culture, we observed an homogenous cells repartition in hydrogels layers separated by polyelectrolyte multilayers. We also observed, by histology, a proteoglycan and total collagen synthesis in the scaffold after 28, 42 and 56 days of culture. Those results were confirmed with qRT-PCR. Indeed, confocal microscopy gave results that indicate a few pericellular increased of type II and X collagen.
Here we showed, for the first time, that it was possible to induce chondrogenesis from hMSCs embedded in a bilayered scaffold composed of Alg/HA hydrogel and that it can be cultured more than 56 days. In spite of those good results, it appeared that after 28 days of in vitro culture, the matrix synthesis was not significantly increased up to 56 days of culture. However, this method allowed to adapt each layer of the scaffold with hydrogel composition or with growth factors, and to mimic cartilage depth-dependant organization. Indeed, in vivo implantation of this structure, after 28 days of in vitro culture, could give cells the ability to synthesize more extracellular matrix and to restore cartilage integrity.
Acknowledgements: This work was supported by the project ANR06-BLAN-0197-01/CartilSpray, from the “Agence Nationale de la Recherche”, and the “Conseil Regional de Lorraine”.

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SECRETOME ANALYSIS OF MESENCHYMAL STEM CELLS FROM HUMAN UMBILICAL CORD STROME DURING THE CHONDROGENESIS

M.C. Arufe[1,2], A. De la Fuente[1], J. Mateos[3], P. Fernandez[3], E. Rendal[1], S. Diaz[1,2], I. Fuentes[1,2], F.J. De Toro[1,2], F.J. Blanco[1,3]
[1]Osteoarticular and Aging Res. Lab. Cellular Therapy Unit. Ciber-BBN. INIBIC-Complejo Hosp. Univ. A Coruña, A Coruña, Spain; [2]Dept. Med. Area of Anatomy and Human Embryology. Fac. Hlth.Sci. Univ. of A Coruña, A Coruña, Spain; [3]Osteoarticular and Aging Res. Lab. Proteomic Unit-Associated Node to ProteoRed. INIBIC-Complejo Hosp. Univ. A Coruña, A Coruña, Spain

Purpose: Our goal is study the secreted proteins of Mesenchymal Stem Cells -MSCs- from umbilical cord stroma during their differentiation towards chondrocyte-like cells.
Methods: Umbilical cord tissues were obtained from caesareans from normal women in the Maternity Facility at Complejo Hospitalario Universitario de A Coruña under the supervision of the hospital ethic committee. We isolated and growth MSCs from umbilical cord stromal tissue by enzymatic digestion and cultured the cells adhered to the plastic plate (1). The cells were characterized (2) using flow cytometry, immunohistochemistry and RT-PCR techniques. Chondrogenic process was performed using our previously published model (3). Briefly, we grow the cells during two days in medium suplemented with FCS 10%. After 2 days we washed the cells with PBS and added medium supplemented with KO serum and TGF-β3. Spheroids were formed by two days in culture and this three-dimensional structure help to produce the characteristic proteins which form part of the extracellular matrix of the cartilage. The spheroid were situated in a well with the chondrogenic factors plus RPMI during 12 h to recover the protein secreted to the medium by the spheroids at 4, 7, 14, 28 and 46 days of differentiation avoiding the serum contamination. SDS-PAGE was done using pre-cast NuPAGE® NOVEX gels (Invitrogen) to ensure reproducibility in the separation of the proteins. Gels were stained with Silver Nitrate and the entire lane was divided in 16 sections. Each section was excised and subjected to in-gel digestion with trypsin. The peptide mixture was cleaned